

**Amendments to the Specification:**

Please amend the specification as shown:

Please replace paragraph [0001] with the following amended paragraph:

[0001] This application claims priority from copending PCT application PCT/US2004/030986 filed September 9, 2004 and U.S. 60/505,264 filed September 22, 2003. Methods and compositions of multiple splice variants of *IG20* are useful to regulate cell death and replication.

Please replace paragraph [00019] with the following amended paragraph:

[0019] A novel human gene (~~IG20~~) (IG20) encodes multiple splice variants. The gene is essential for survival of the animal (knockout mice die immediately after birth). Splice variants have very important biological functions, but differ in their ability to affect cell death, survival and replication. The-IG20 splice variant is pro-apoptotic, anti-proliferative, and renders cells more susceptible to induced cell death (i.e. is a tumor suppressor).

Please replace paragraph [00033] with the following amended paragraph:

[0033] Various methods of treatment that can lead to over-expression of ~~IG20~~ the IG20 gene or cDNA expression and prevention of splicing that results in larger amounts of IG20 accumulation in the cell are disclosed. A method to selectively prevent splicing of ~~IG20~~ IG20 is performed with anti-sense oligos directed against the splice region or through manipulation of other unidentified ~~IG20~~ IG20 splice factors. This can also be accomplished either through transfection of the whole, or portions of the protein/peptides. A similar effect can be accomplished through silencing of DENN-SV using Si RNA, anti-sense RNA, other oligonucleotides, DENN-SV protein or its fragments that can act as dominant negatives, and the like. Results described herein show the use of DENN-SV to facilitate cell survival and growth of primary cells such as beta cells in the islet of Langerhans (insulin producing cells), neuronal cells, stem cells, and the like.

Please replace paragraph [0046] with the following amended paragraph:

[0046] **FIG. 6: Effects of Dominant Negative-I $\kappa$ B and CrmA on TNF- $\alpha$  induced, IG20-mediated apoptosis.** Blocking effects of DN $\kappa$ B $\alpha$  and CrmA on the pro-apoptotic and anti-apoptotic effects of IG20 and DENN-SV are shown. Percentages represent mitochondrial

depolarization (used as an indicator of apoptosis) due to TNF- $\alpha$  and cyclohexamide treatment; this was calculated by subtracting percentage of TMRE-negative cells transfected with either empty vector or with vectors containing either CrmA or DN $\kappa$ B $\alpha$ , from percentage of TMRE-negative cells transfected with the same construct ~~but treated~~ but treated with TNF- $\alpha$  and cyclohexamide. TMRE data were obtained from GFP-positive gated cells only. Data shown in the figure represent three different wells for each sample. Experiment was repeated at least three times and consistent results were obtained. P values were  $< 0.05$  for all test groups.

Please replace paragraph [0078] with the following amended paragraph:

[0078] The ~~IG-20~~ IG20 gene is essential for survival of animals. It is over-expressed in human tumors and cancer cell lines, and can encode 4 different splice variants. The DENN-SV splice variant is constitutively expressed in all cells and tissues, and is highly-expressed in human tumors and cancer cell lines relative to normal tissues and other splice variants. Cells transfected with a cDNA encoding DENN-SV proliferate more aggressively, form larger colonies in soft agar and become resistant to TNF- $\alpha$ , TRAIL, etoposide and vinblastine induced cell death. In contrast, cells transfected with a cDNA encoding IG20 splice variant become more susceptible to cell death induced by the above treatments and grow slowly in culture. In addition, the increased susceptibility of IG20 transfected cells to TNF- $\alpha$  and TRAIL induced death is mediated by the activation of caspases-8 and -3 resulting from enhanced recruitment of caspase 8 and FADD to the Death Inducing Signaling Complex (DISC). The other two splice variants, MADD and IG20-SV2, exhibit little or no effect. More interestingly, cells that lack IG20, such as PA-1-ovarian carcinoma cell line, proliferate rapidly and resist TRAIL induced apoptosis. However, after IG20 is introduced, they replicate slowly and become susceptible to TRAIL induced apoptosis. These observations clearly show that DENN-SV and IG20 are biologically very important. Differential expression of IG20 and DENN-SV splice variants renders cells either more susceptible or resistant to induced cell death respectively, and the pro-apoptotic property of IG20 variant can be exploited to render tumor cells that are otherwise resistant to become susceptible to killing by TRAIL and/or chemotherapeutic agents.

Please replace paragraph [00121] with the following amended paragraph:

**[0121] HeLa IG20 cells show increased activation of initiator and effector caspases -**

Addition of TRAIL results in receptor clustering, which facilitates FADD and caspase-8 recruitment leading to effector caspase-3 activation. In order to analyze whether IG20 mediated ~~itself~~ its effects by increasing activation of caspases, levels of caspase-8, were tested which is the main activator caspase involved in TRAIL mediated death pathway. Caspase-10, a molecule with sequence similarity to caspase-8 has been shown to participate with caspase-8 in the DR4 and DR5 signaling pathways and therefore their levels in TRAIL treated cells were determined. As seen in FIG. 15A, the levels of both caspases, measured in fluorescence intensity, increased in TRAIL treated HeLa IG20 cells compared to TRAIL treated control cells. Cleavage of Pro-Caspase-8 results in active caspase-8 and a 10 kDa fragment (p10) that can be readily detected in a western blot using a p10 specific antibody. An increase in the amount of cleaved caspase-8 is seen in HeLa IG20 cells relative to the control cells tested at different timepoints after TRAIL treatment (FIG. 15B). The blot was also stripped and reprobed with anti- $\beta$ -actin to ensure equal protein loading.